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(54) Title: PEPTIDE-BASED VACCINE AGAINST PAPILLOMAVIRUS INFECTION

(57) Abstract

Peptide-based vaccine against papillomavirus infection is disclosed. The immunizing component of the vaccine is at least one peptide selected from the following groups of peptides a) to e): a) Ala Thr Val Tyr Leu Pro Pro Val Pro Val Ser Lys Val Val Ser and some specified analogs thereof, b) Gln Pro Leu Gly Val Gly Ile Ser Gly His Pro Leu Leu Asn Lys Leu Asp Asp Thr Glu and some specified analogs thereof, c) Lys Gly Ser Pro Cys Thr Asn Val Ala Val Asn Pro Gly Asp Cys Pro Pro Leu Glu Leu and some specified analogs thereof, d) Arg Ala Gly Thr Val Gly Glu Asn Val Pro Asp Asp Leu Tyr Ile and some specified analogs thereof, and e) Ser Thr Ile Leu Glu Asp Trp Asn Phe Gly Leu Gln Pro Pro Pro Gly Gly Thr Leu Glu and some specified analogs thereof.

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PEPTIDE-BASED VACCINE AGAINST PAPILLOMAVIRUS INFECTION

The present invention relates to a peptide-based vaccine against papillomavirus infection.

Background

The human papillomaviruses (HPVs) cause a variety of proliferative epithelial lesions, from common warts to premalignant intraepithelial neoplasias of the anogenital region (zur Hausen, 1991). Thus far, 68 different types of human papillomavirus (HPV) have been isolated (de Villiers, 1992). HPV type 16 is the predominant type found in human anogenital cancers and high-grade intraepithelial neoplasias (Lorinoz et al., 1992). The HPV capsid contains two proteins encoded by the L1 and L2 open reading frames (ORFs). The major protein of the PV capsid is an approximately 57 kDa protein encoded by L1 (Li et al., 1987); Pliacinski et al., 1984; Tomita et al., 1987). The L2 ORF codes for a 76 kDa protein which is the minor structural protein of the PV capsid (Komly et al., 1986). The HPV virion consists of an icosahedral capsid with 72 capsomeres composed of pentamers of the major capsid protein L1 (Baker et al., 1991). The function and structural location of the minor capsid protein L2 is unclear.

Neutralizing monoclonal antibodies against cottontail rabbit papillomavirus (CRPV) (Christensen & Kreider, 1991), bovine papillomavirus (BPV) (Christensen & Kreider, 1993) and HPV11 (Christensen et al., 1990) have hitherto only been generated against conformation-dependent epitopes on intact virions and it has not been possible to map their location due to the conformational dependence of these epitopes.

Immunization with an L2 protein of CRPV induces low-titered neutralizing antibodies, indicating that at least part of L2 is exposed on the virion surface (Christensen et al., 1991).

However, no specific sequences have been identified which are exposed on the virion surface of HPV. It was hypothesized that intact virions may contain surface-exposed linear epitopes which could be identified by antibodies generated by immunization with peptides. A knowledge of the specific amino acid sequences of antigenic sites - exposed on intact virus particles and present in neutralizing epitopes - should enable design of effective immunogenes for vaccination.

It is well recognized that the structure of neutralizing epitopes with the ability of conferring protection is an essential requirement for the design of effective vaccines. Extensive attempts have been made to identify such neutralizing epitopes, however, without success. The problems are: 1) Immunization with an intact capsid containing conformationally sensitive epitopes may induce mostly antibodies to conformational epitopes which cannot be mapped for practical reasons. 2) Immunization with a denatured whole capsid protein will not induce antibodies against conformationally sensitive epitopes, but antibodies are preferentially made against immunodominant epitopes which are found at the inside of the capsid and these epitopes are thus not useful for vaccination.

Since it has not been possible to experimentally define the surface-exposed neutralizing epitopes, previous attempts to identify surface-exposed epitopes had to rely on computer algorithms that may identify evolutionarily variable or hydrophilic amino acid stretches which might conceivably be surface-exposed. Such algorithms have been unreliable, since they have either not worked or have only had a limited success.

Description of the invention

In the experimental work, on which the present invention is based, antisera against 77 overlapping synthetic peptides from the L1 and L2 proteins of HPV16 were used to generate antipeptide antisera in guinea pigs. The resulting hyperimmune sera were tested for reactivity with intact HPV16 particles in order to identify surface-exposed epitopes. Finally, antisera against synthetic peptides from the

corresponding surface-exposed sites of rabbit papillomavirus were tested for their ability to neutralize infectious rabbit papillomavirus.

A hypothesis that was made was the proposal that it should be possible to bypass the problems caused by immunodominant epitopes (either of conformational nature or located at the inside of the virus) by empiric and systematic testing of synthetic peptide immunogens for their ability to induce antibodies which are reactive with the intact virus and, in a second step, test the ability of such antibodies to neutralize infectious virus.

Some of the peptides used in the present invention have previously been described to be antigenic (WO 90/04790 and WO 91/18294), but the possibility that their antigenicity could be exploited to experimentally define surface-exposed neutralizing epitopes useful for protection against papillomavirus infection was not indicated. Nor was it suggested that a systematic synthesis and testing of analogs would result in identification of peptides with considerably improved immunogenicity. As detailed below, the exact amino acid sequence was found to be critical for the immunogenic properties of the peptides.

Thus, the present invention is directed to a vaccine against papillomavirus infection, which comprises as an immunizing component at least one peptide selected from the following groups of peptides a) to e).

a)

Ala Thr Val Tyr Leu Pro Pro Val Pro Val Ser Lys Val Val Ser

5 10 15

wherein Ala in position 1 may be substituted for Asn, Gly, Ser, or Thr,
Thr in position 2 may be substituted for Lys, Leu, Met or Gln,
Val in position 3 may be substitutde for Leu or Phe,
Leu in position 5 may be substituted for Val,
Val in position 8 may be substituted for Thr, Pro, Gln, Ala, or for the two amino-acid residues Pro Ala or Pro Asn,
Pro in position 9 may be substituted for Ser,

Ser in position 11 may be substituted for Ala or Thr,
Lys in position 12 may be substituted for Arg or Thr,
Val in position 13 may be substituted for Ile, Leu or omitted,
Val in position 14 may be substituted for Ile, Leu or Pro,
Ser in position 15 may be substituted for Asn, Ala, Pro or Thr,

b)

Gln Pro Leu Gly Val Gly Ile Ser Gly His Pro Leu Leu Asn Lys Leu Asp Asp Thr Glu
5 10 15 20

wherein Val in position 5 may be substituted for Leu, Ile or Ala,
Ile in position 7 may be substituted for any other amino-acid residue,
His in position 10 may be substituted for Asn,
Leu in position 12 may be substituted for Tyr or Phe,
Lys in position 15 may be substituted for Arg,
Leu in position 16 may be substituted for Gln, Tyr, Asp or Phe,
Asp in position 17 may be substituted for Glu or Asn,
Thr in position 19 may be substituted for Val,

c)

Lys Gly Ser Pro Cys Thr Asn Val Ala Val Asn Pro Gly Asp Cys Pro Pro Leu Glu Leu
5 10 15 20

wherein Lys, Gly and Ser in positions 1,2 and 3, respectively, may be individually omitted,
Pro in position 4 may be substituted for Leu, Ala, Val, Thr, Ile, Ser or Gln,
Cys in position 5 may be substituted for Ser,
Thr in position 6 may be substituted for Lys, Arg, Ala, Asn, Ser, or Gly,
Asn in position 7 may be substituted for Pro, Gln, Glu, Asn, Arg, Ser, Ala or Thr
Val in position 8 may be substituted for Asn, Asp, Pro, Thr, Ser, Ala, Arg or Gly,
Ala in position 9 may be substituted for Thr, Pro, Ser, Asn, Gln, Lys or Arg,
Val in position 10 may be substituted for Leu, Gln, Ser, Thr, Pro, Gly, Ile or omitted,
Asn in position 11 may be substituted for Ala, Ser, Gln, Thr, Arg, Pro, Val,

Lys or omitted,

Pro in position 12 may be substituted for Ala, Asn, Gln,, Val, Arg, Thr, Leu, Asp, Ser or omitted,

Asp in position 14 may be substituted for Glu,

Cys, Pro, Pro, Leu, Glu and Leu in positions 15, 16, 17, 18, 19 and 20, respectively, may be individually omitted,

d)

Arg Ala Gly Thr Val Gly Glu Asn Val Pro Asp Asp Leu Tyr Ile
5 10 15

wherein Arg in position 1 may be substituted for Lys or Leu,

Ala in position 2 may be substituted for Gln, Gly, Leu or Ser,

Gly in position 3 may be substituted for Ser or Val,

Thr in position 4 may be substituted for Ala, Met, Val, Asp, Lys, Ser, Gly, Asn or Glu,

Val in position 5 may be substituted for Met, Leu, Ile, Ala or Thr,

Glu in position 7 may be substituted for Asp,

Asn in position 8 may be substituted for Pro, Thr, Ala, Lys, Asp, Gln, Glu or Ser,

Val in position 9 may be substituted for Ile or Leu,

Asp in position 11 may be substituted for Asn, Glu, Thr, Ser, Ala, Gln or Gly,

Asp in position 12 may be substituted for Glu, Ala, Thr, Ser or Gln,

Leu in position 13 may be substituted for Met, Phe or Tyr,

Tyr in position 14 may be substituted for Ile, Leu, Val or Met,

Ile in position 15 may be substituted for Thr, Leu, Ile, Val, Trp or Phe,

and

e)

Ser Thr Ile Leu Glu Asp Trp Asn Phe Gly Leu Gln Pro Pro Pro Gly Gly Thr Leu Glu
5 10 15 20

wherein Ser in position 1 may be substituted for Pro, Asn, Lys, Ala or Thr,

Thr in position 2 may be substituted for Asp, Asn, Ser, Ala, Gln, Arg or Gly,

Ile in position 3 may be substituted for Leu,

Leu in position 4 may be substituted for Ile,
Glu in position 5 may be substituted for Asp,
Asp in position 6 may be substituted for Glu, Gln, Gly or Asn,
Asn in position 8 may be substituted for Gln,
Phe in position 9 may be substituted for Val or Ile,
Gly in position 10 may be substituted for Ala, Ser or Lys,
Leu in position 11 may be substituted for Ile or Val,
Gln in position 12 may be substituted for Thr, Ala, Val, Pro, Ser or Gly,
Pro in position 13 may be substituted for Ala, Leu or Thr,
Pro in position 14 may be substituted for Ala or Val,
Pro in position 15 may be substituted for Gln,
Gly in position 16 may be substituted for Ser, Thr, Ala or Asn,
Gly in position 17 may be substituted for Ser, Thr or Ala,
Thr in position 18 may be substituted for Ser,
Glu in position 20 may be substituted for Gln or Val.

It is possible that the above listed peptides can be subject to some modifications, such as extensions, deletions and substitutions without losing their immunzing properties, and the present invention is intended to comprise such equivalent modified peptides.

In an embodiment of the invention the vaccine comprises at least two immunizing components selected from at least two different groups of peptides a) to e).

In a preferred embodiment of the invention the
immunizing component(s) is (are) chosen from the peptides
a)
Ala Thr Val Tyr Leu Pro Pro Val Pro Val Ser Lys Val Val Ser
5 10 15

b)
Gln Pro Leu Gly Val Gly Ile Ser Gly His Pro Leu Leu Asn Lys Leu Asp Asp Thr Glu
5 10 15 20

c)

Lys Gly Ser Pro Cys Thr Asn Val Ala Val Asn Pro Gly Asp Cys Pro Pro Leu Glu Leu
5 10 15 20

c1)

Pro Ser Thr Asn Val Ala Val Asn Pro Gly Asp
5 10

d)

Arg Ala Gly Thr Val Gly Glu Asn Val Pro Asp Asp Leu Tyr Ile
5 10 15

e)

Ser Thr Ile Leu Glu Asp Trp Asn Phe Gly Leu Gln Pro Pro Pro Gly Gly Thr Leu Glu
5 10 15 20

Suitably said peptide(s) is (are) coupled to a carrier or is (are) multimer forms of the peptide(s).

Examples of carriers that may be used, and have been used, are keyhole limpet hemocyanin and tetanus toxoid. Multimer forms of the peptides may be produced by coupling the peptides to e.g. several inter-connected lysines.

As is well known in the art, the effects of a vaccine may be enhanced by the use of an adjuvant. Therefore, the vaccine according to the invention may additionally comprise an adjuvant.

Description of the drawings

Fig 1 shows Immunoreactivity of the antipeptide antisera against (a) HPV16 L1 and (b) HPV16 L2 with intact (open bars) or disrupted (filled bars) HPV16 VLPs. Each column represents the ELISA absorbance value (OD) for one antiserum against L1 and L2 peptides reacted with the HPV16 VLPs. The number of each peptide is given below the abscissa. Peptide HPV16 L1 (L2:1) corresponds to the amino terminus of the L1 (L2) and the peptide L1:35 (L2:66) to the carboxy-terminus of the protein.

Fig 2 shows the results obtained when intact or disrupted HPV16 capsids (VLPs) were added in serial dilutions to ELISA plates coated with antibodies against peptide L1:20 and bound virus detected with antibodies against peptide L1:20 or peptide L1:29.

Next, the experimental work is described.

Material and Methods

Peptides derived from the L1 and L2 OFR:s of HPV16 were synthesized by the solid phase method as described previously (Dillner et al., 1990, and the international patent applications Nos WO 90/04790 and WO 91/18294). The peptides were conjugated to the carrier protein keyhole limpet hemocyanin and used for immunization of guinea pigs as described (Dillner et al., 1991). When tested in peptide ELISAs 56 % of the immunized guinea pigs had responded with antipeptide titers of 1:10000 or more and only 5 % of the immunized guinea pigs failed to respond. In experiments where the peptides were cysteine-containing they were conjugated to tetanus toxoid using the bromoacetic acid N-hydroxysuccinimide ester (Askelöf et al., 1990) whereas the other peptides were conjugated using glutaraldehyde (Dillner et al., 1991).

Capsid antigen detection. The antisera were tested by the peroxidase-antiperoxidase (PAP) staining method on formalin-fixed, paraffin-embedded sections of cervical condylomas, cervical intraepithelial neoplasia (CIN) lesions and common warts. Four µm thick sections were deparaffinized, rehydrated and treated with 3 % hydrogen peroxide in PBS. After blocking with 5 % milk for 60 min the sections were incubated with antipeptide antisera, diluted 1:1000. After intermediate PBS washes the sections were incubated for 1 h with anti-guinea pig immunoglobulins (Dako, Copenhagen, Denmark), diluted 1:150, for 1 h with swine anti-rabbit immunoglobulins (Dako), diluted 1:100, and for 1 h with a rabbit peroxidase-antiperoxidase complex (Dako), diluted 1:100. After a final PBS wash the antibody complex was visualized by the addition of 3-amino-ethylcarbazole in acetate buffer (pH 4). Counter staining was

performed with Mayer's hematoxylin for 15-30 s. The first and last section of each block were up to 1991 stained with a commercially available rabbit antiserum raised against SDS-treated BPV type 1 (Dako) and then the monospecific antiserum 16a (Dillner, et al., 1991) was used. These antisera detect PV group-specific antigens on the capsid protein and were used as a positive control.

ELISA. Guinea pig and rabbit antisera against 77 synthetic peptides derived from the L1 and L2 ORFs of HPV16 were tested for reactivity in ELISA against intact and disrupted HPV16 capsids. For disruption the capsids were diluted to 1 µg/ml in 0.1 M carbonate buffer (pH 9.6) and kept at room temperature (RT) for 4 h before coating onto ELISA plates. For analysis of intact capsids the dilution was performed in phosphate buffered saline (PBS) immediately before coating. Fifty µl of the dilution was added to each well of a microtiter plate (Costar, Cambridge, MA, USA) and the plates were incubated at +4°C overnight. After 2 washes with ice-cold PBS the plates were blocked with 10 % horse serum (heat-inactivated; Sigma, S:t Louis, MO, USA) in PBS (HS-PBS) for 1 h. After discarding the blocking solution, antipeptide antisera (diluted 1:50 or 1:100 in HS-PBS) were added to the plates which were allowed to react for 2 h. After 5 washes with PBS-0.1% Tween 20 (PBS-T), peroxidase-conjugated rabbit anti-guinea pig immunoglobulins, diluted 1:2000, or goat anti-rabbit immunoglobulins, diluted 1:1000 (Dako, Copenhagen, Denmark) in HS-PBS were added to the plates which were incubated at room temperature for 2 h. After 5 washes, the plates were developed with 0.4 mg/ml of 2.2'-azino-di(3-ethylbenz-thiazolinsulfonate)deammonium salt in 0.1 M citrate buffer (pH 4) containing 0.9 % hydrogen peroxide and the A_{415} -values were recorded after 15 minutes.

For two-site ("catching antibody") ELISA, the plates were coated at +4°C overnight with the IgG fraction of rabbit antipeptide antiserum diluted 1:30 in PBS. After one wash with PBS-T and blocking with HS-PBS for 1 h the plates were incubated at 37°C for 2 h with serial dilutions of capsids diluted either in PBS (intact particles) or in 0.1 M carbonate buffer (pH 9.6) (disrupted particles). After 5 washes with PBS-T the plates were incubated for 1 h at 37°C with guinea pig antipeptide antisera, diluted 1:100 in HS-PB. After 5 washes with PBS-T, peroxidase-conjugated guinea pig

immunoglobulins, diluted 1:1000 (Dako, Copenhagen, Denmark) in 8 % horse serum and 2 % rabbit serum in PBS, were added to the plates which were incubated at 37°C for 45 min. Development and recording of the color reaction were as above.

Generation of HPV16 capsids:

Cloning of the capsid protein gene of a new wild-type isolate of HPV type 16.

A cervical swab sample was obtained from a healthy Swedish woman, previously determined to be infected with HPV type 16 (sample provided by Dr. Lena Dillner, Dept. of Virology, Karolinska Institute). The HPV16 L1 coding sequence was amplified by means of the polymerase chain reaction (PCR). The amplified fragment was ligated blunt end into the SmaI-digested calf intestinal alkaline phosphatase (CIAP) treated SFV expression vector, pSFV1 (kindly provided by H. Garoff and P. Liljeström, Novum, Huddinge, Sweden), a procedure which resulted in the plasmid pSFV16L1, or into EcoRV restricted CIAP treated pBluescript, a procedure which resulted in pT7-16L1. The plasmid pSFV16L1 was linearized with SpeI and capped RNA was synthesized in vitro at 37°C for 1 h in a total volume of 50 µl of SP6 buffer (40 mM Hepes-KOH [pH 7.4], 6 mM MgOAc, 2 mM spermidine-HCl, 5 mM DTT, 1 mM m^7(G5')ppp(5')G, 0.5 mM GTP, 1mM CTP, 1 mM UTP, 1 mM ATP, 50 U Rnasin and 30 U SP6 RNA polymerase. About 10^7 baby hamster kidney cells (BHK-21) were trypsinized and washed once with PBS. Twenty to 25 µl of each recombinant RNA was used to electroporate 0.8 million cells. The cells were pulsed twice at 850 V/25 µF at room temperature. The transfected cells were resuspended in 24 ml of BHK-21 medium containing 5 % fetal calf serum, 10 % tryptose phosphate broth, 20 mM Hepes, 2 mM glutamine, 0.1 U/ml penicillin, 0.1 µg/ml streptomycin, and plated onto eight 35 mm cell culture plates. Cells were harvested 24 to 48 h posttransfection. For production of recombinant SFV capped RNA transcribed from pSFV15L1 or pSFV3-lacZ was electroporated into BHK-21 cells together with an equal amount of RNA produced from the helper plasmid pSFV-Helper1. Medium containing recombinant SFV (vSFV16L1 or vSFV3-lacZ) was either used for infection of BHK-21 cells directly or stored at -70°C until used.

For infection of BHK-21 cells recombinant virus was first diluted 1:10 in minimal essential medium (MEM) containing 0.2 % bovine serum albumin, 2 mM glutamine and 20 mM Hepes. A total volume of 500 µl of virus suspension was used to infect a 35 mm plate with BHK-21 cells. After 60 min, the medium containing virus was removed, 3 ml of BHK-21 medium was added to each plate and the incubation was allowed to continue for 24 to 48 h.

The cells were harvested by scraping 24 to 48 h postinfection and centrifuged at 600 x g for 5 min, washed once with ice-cold PBS and stored at -70°C. Thawed cell pellets were resuspended in 10 ml of ice-cold PBS and sonicated on ice for 3 x 30 s. The lysates were then layered on cushions of 40 % (wt/vol) sucrose-PBS and centrifuged for 2.5 h at 25,000 rpm in an W-28 rotor. The pellets were resuspended in 8 ml of PBS containing 27 % (wt/wt) CsCl, transferred into quick-seal tubes and centrifuged in an SW28 rotor for 20 to 22 h at 28,000 rpm. Ten fractions were collected from each CsCl gradient and dialysed against PBS overnight. The fractions were analyzed for presence of HPV16 capsids by means of Western blotting and two-site ELISA. The fractions containing capsid proteins were also analyzed by electron microscopy and found to contain typical papillomavirus-like particles. The results are presented in Figures 1 and 2.

Epitopes displayed in sections of HPV-infected tissue.

One hundred and fourteen cervical biopsy specimens, mostly from low-grade dysplasias and condylomas, positive for HPV6, 11, 16, 18, 31, 33 or 35 as well as 2 HPV-negative specimens were tested for expression of the HPV capsid antigen by immunostaining with an antiserum against PBV (Dako) and/or an antipeptide antiserum against an HPV group-specific epitope (Dillner, et al., 1991). Seven specimens could not be tested due to the poor quality of the sections or the very small amounts of epithelium present. Fifty two out of 107 cervical lesions (49%) were found to express the capsid antigen. For 28 out of 52 capsid antigen positive biopsies it was only possible to cut multiple consecutive sections where both the first

and the last section were positive for the HPV capsid antigen. Thirty five consecutive sections from six biopsies were cut and tested with antisera to 35 peptides corresponding to the entire major capsid protein L1 (Table 1a). Five biopsies were from cervical condylomas or CIN lesions positive for HPV6, 16, 16/18, 31 or 33 and one was from a cutaneous wart. Antisera exhibiting a nuclear staining with some of the six biopsies were further tested with one additional biopsy specimen from a skin wart and six additional biopsies from cervical lesions positive for HPV 6/11, 18 or 35, 31/33/35. Stained histological sections of cervical lesions HPV-negative with our antipeptide antisera from HPV16 L1 and L2 ORF:s were used as controls. No staining was observed. Furthermore, histological sections containing HPV capsid proteins were tested with preimmune sera and no staining was detectable in the cells containing capsid antigen. Eleven of the 35 antipeptide antisera gave a specific nuclear staining in cells also positive when stained with group specific antisera for PV and in the same area of the epithelia that contained HPV-DNA when tested by ISH. Some epitopes seem to be shared by most human papillomavirus types, e.g. peptides number L1:13 and L1:16 (at the positions 182-201 and 227-246, respectively) since they were exhibited also in the HPV capsid antigens found in cutaneous warts. Most antisera only stained the genital type of HPV capsids, but one of the l1 antisera gave a type specific staining for HPV16. The number of positive cells and the intensity of the immunostainings varied with the different antipeptide sera. Strong staining intensities were mostly obtained with antisera against peptides number 13, 16, 20 and 22. The strongest staining intensities were consistently given by the antisera against the peptides 16a and 16, which were also the most cross-reactive antipeptide sera.

Thirty-one sections from two specimens, one from a HPV16 positive lesion and the other from a HPV33 positive dysplasia, were tested with 31 antisera against peptides corresponding to the entire L2 protein. Seven of the antipeptide antisera (against the peptides 38, 44, 46, 60, 61, 62 and 63;) gave a specific nuclear staining in the virusproducing tissue. Sections from 15 additional biopsies from cervical lesions infected with HPV 6, 11, 16, 18, 31, 33 and/or 35 were tested for reactivity with the seven reactive antisera. The strongest staining and the highest number of stained

nuclei was obtained with antisera against the peptides L2:44, L2:46 and L2:60. The antipeptide antisera against the peptides 38, 44 and 46 stained the HPV capsid antigen of several different genital types of HPV whereas the antiserum against the peptide 60 only reacted with HPV16 and HPV33 positive lesions, and the antisera against peptides L2:61; L2:62 and L2:63 only reacted with HPV16 positive specimen(s).

Epitopes exposed on intact or disrupted HPV16 capsids.

All antipeptide antibodies were screened in ELISA for reactivity with intact and disrupted HPV16 and BPV capsids as well as native and denatured HPV11 particles at a dilution of 1:100. Antisera against 19 peptides from HPV16 L1 reacted with intact HPV16 capsids (Fig 1a). Most of the reactive sera had also been reactive with the PV capsid antigen in immunohistocytochemical stainings. Antisera against 12 peptides were also reactive with disrupted virus (Fig 1a). Some sera, e.g. against the peptides 8, 10, 14, 16 and 29, reacted roughly as well with disrupted as with intact particles (Fig 1a). Three antipeptide antisera (number 3, 20 and 31 were much more reactive with intact than with disrupted particles (Fig 1a). All 31 antisera against peptides corresponding to the L2 protein of HPV16 were also tested for reactivity with HPV16 capsids. Seven antipeptide antisera reacted with intact HPV16 capsids (Fig 1b). Preimmunization serum samples from the same animals tested in parallel with the postimmunization serum samples. Weak reactivity (<30% the postimmunization serum samples) was detected in the serum of one non-immunized animal whereas no other serum samples from non-immunized animals showed any detectable reactivity (ELISA OD < 0.050).

The five antisera exhibiting the strongest reactivity with intact HPV16 capsids as well as a rabbit antiserum to one of these peptides were titrated in serial 3-fold dilutions for reactivity against the immunizing peptides and intact HPV16 capsids. Two guinea pig antisera and one rabbit serum were reactive at a 1:146 000 titer (Table 2). Interestingly, two guinea pig antisera had an identical titer against HPV16 capsids and against the immunizing peptide (Table 2), which indicates that these synthetic

immunogens mostly or exclusively induce antibodies reactive with intact HPV16 capsids.

Verification of epitope exposure on non-disrupted virions by two-site ELISA.

Exposure to a carbonate buffer of high pH is the original method used for disrupting papillomaviruses in many classical studies. In order to quantify the disruption efficiency and exclude the possibility that the reactivity with native capsids might have been due to contaminating monomers, a catching antibody ELISA was used. ELISA plates were coated with antibodies against the peptide L1:20 and then capsids, diluted either in carbonate buffer or in PBS were added. Subsequently, antipeptide antisera against the same or other L1 peptides were allowed to react with the particles bound to the first antibody. As shown in Fig 2, virus diluted in PBS could bind two antibodies directed against the same site, a result which demonstrates that the virus exists as multimers of the capsid protein and confirms that the L1:20 epitope indeed is exposed on intact virions. In contrast, virus diluted in carbonate buffer could only bind the L1:20 antibodies once, a result which indicates that the virus was disrupted into monomers of the capsid protein. The inability to detect bound capsids is not due to destruction of the L1:20 epitope in carbonate buffer, since it was possible to bind the capsid protein to the coated anti-L1:20 antibodies and subsequently detect it with antibodies against another antigenic site (Fig 2).

Optimization of peptide immunogenicity.

Improvement of the immunogenicity of the 4 most useful peptide immunogens was attempted by synthesizing peptide analogs and raising hyperimmune sera in guinea pigs against these peptide analogs. Tetanus toxoid (TT) was from now on used as carrier protein instead of keyhole limpet hemocyanin as TT is a carrier protein suitable for human use. The titer definition is half the maximal ELISA absorbance. The results are presented in Table 3.

For L1:3 the original peptide gave considerably better results when conjugated to TT than when conjugated to KLH. When 3 residues were removed at the C-terminal end, 3 residues at the N-terminal end and the conjugation was performed via an exogenous cysteine the same results were obtained as with the original peptide. (Compare peptides A71-23 and A71-13). However, among the various peptide modifications tried the best titers were obtained if a carbon spacer (aminobutyric acid) was added to the amino terminus (peptide A71-26).

For L1:29 addition of various spacers had very little effect, but the deletion of 2 amino terminal residues impaired the immunogenicity. Thus far, the best results have been obtained with the original peptide.

For L1:20 addition of spacers again had very little effect. Addition of cysteine to the amino terminal or to the carboxy terminal ends of the peptide was performed in order to investigate if cysteine specific conjugation would represent an improvement. Omission of 2 residues in combination with a cysteine specific conjugation via a carboxy terminal cysteine residue resulted in an equally effective immunogen as the original peptide. On theoretical grounds, this peptide (A71-17) was considered the best immunogen.

For L1:14, the presence of 2 cysteine residues was considered suboptimal for cysteine-specific conjugation, and one of the cysteine residues was thus replaced by a serine residue. Both the serine replacement analogs worked well as immunogens, the one with an N-terminal serine replacement (A71-15) being somewhat better (not evident in Table 3). Three truncation analogs were made all of which had a serine replacement similar to that of A71-15. A peptide of 15-residues (A76-12) represented a considerable improvement in immunogenicity and is considered to be the best possible immunogen for this antigenic site. It is noteworthy that also a very short peptide, the peptide of 12-residues A76-14, was highly immunogenic.

Selection of peptides likely to work as immunogens for eliciting antibodies against intact virus particles also of other papillomaviruses.

Since the morphological structure of the papillomavirus capsid is very similar for all papillomaviruses and since there is considerable amino acid homology in the capsid protein gene of all papillomaviruses, we hypothesized that the corresponding sites would also be surface exposed and antigenic for other papillomaviruses. Several alignments for maximal homology between papillomavirus capsids proteins have been published. Selected areas of homology to the known surface-exposed sites are shown in Table 4. Accordingly, the homology in the HPV capsid protein is sufficiently strong to unambiguously perform an alignment in order to identify the maximally homologous region to the HPV16 surface exposed sites. In the case of the CRPV homologue of L1:14, the aminoterminal cysteine residue was also substituted with a serine residue and the 4 carboxy terminal residues were truncated, which resulted in a CRPV peptide that was maximally homologous with the best L1:14 analog in HPV 16 (peptide A76-12). As shown in Table 5, all four selected CRPV peptides (and also one BPV and one HPV11 peptide) induced antibodies reactive with the intact CRPV capside.

Antipeptide antibodies confer protection against papillomavirus infection.

Three of the antipeptide antisera reactive with intact CRPV (number A71-2, A71-8 and A71-11) were then evaluated with respect to their ability to neutralize infectious CRPV by Dr. Francoise Breitburd. Unite des Papillomavirus, Institut Pasteur, Paris, France. The antipeptide antisera were incubated at a 1:2 dilution for 1 h at 37°C with an infectious dose of CRPV titrated to give rise to 10-20 papillomas and then inoculated into a group of six rabbits. Pre-immunization bleeds obtained from the same animals, before peptide immunization were used as controls. Papillomas will typically appear within a few weeks post inoculation. Two antipeptide antisera (A71-2 and A71-8) were found to confer complete protection. No papillomas were seen even 3 months post inoculation. The third antiserum (A71-11) conferred partial protection. One transient papilloma was seen 3 weeks post inoculation.

Conclusions

The delineation of the antigenic structure of the HPV capsid is important for several reasons. The availability of a panel of site-directed antisera against epitopes exposed on HPV particles could be important for studies of the expression of the HPV capsid during the viral life cycle, for studies of viral infectivity and contagiousness and will enable HPV detection and typing by simple immunological techniques such as immunoperoxidase staining or two-site ELISA. Several antisera were found that showed a strong, type-restricted staining. In particular, the strongly stained L2 epitope L2:60 was found only in tissue infected by HPV16 and the closely related HPV33.

The location of the L2 protein in PV capsids is unclear (Baker, et al., 1991). We find that some antisera raised against peptides from HPV16 L2 reacted mainly with intact capsids, confirming that part of the L2 protein is exposed on the surface of the HPV capsid.

The major immunogenic linear epitopes seen by the human immune response in infected individuals has been reported previously (Dillner, et al., 1990, international patent applications Nos. WO 90/04790 and WO 91/18294). Several major immunogenic epitopes (L1:13, L1:14, L1:16 and L1:31) all correspond to major antigenic epitopes, but many of the major antigenic epitopes are poorly reactive or unreactive with human sera.

Above all, knowledge of the antigenic structure of HPV is important for the design of preventive vaccines. The present study has found several synthetic immunogens that induced high-titered responses against intact HPV16 capsids. Furthermore, several of these responses are not type-restricted to HPV16. Among the major epitopes identified L1:14 and L1:20 are promising because of the high titer obtained of the corresponding antisera against HPV16. The epitopes L1:3 and L1:29 are excellent because of their identical titers against the immunizing peptide and against the virus. The present identification and characterization of antigenic sites exposed

on the HPV capsids enabled design of defined immunogens inducing protective antibodies. Thus, the peptides are useful as immunizing components in the vaccines of the present invention.

Table 1

Sequences of the 20 residue peptides overlapping by 5 residues from the HPV 16L1 (a) and L2 protein (b) that were used for immunization

Table 1a

PEPTIDE SEQUENCES OF 20 AA OVERLAPPING PEPTIDES CORRESPONDING TO THE HPV 16L1 PROTEIN

No.	Peptide sequence	Range
1	QVTFIYILVITCYENDVNVY	(2-21)
2	DVNVYHIFFQMSLWLPSEAT	(17-36)
3	PSEATVYLPPVPVSKVVSTD	(32-51)
4	VVSTDEYVARTNIYYHAGTS	(47-66)
5	HAGTSRLLAVGHPYFPIKKP	(62-81)
6	PIKKPPNNNKILVPKVSGLQY	(77-96)
7	SGLQYRVFRIHLPDPNKFGF	(92-111)
8	NKFGFPDTSFYNPDTQRLVW	(107-126)
9	QRLVWACVGVEVGRGQPLGV	(122-141)
10	QPLGVGISGHPLLNKLDDTE	(137-156)
11	LDDTENASAYAANAGVDNRE	(152-171)
12	VDNRECISMDYKQTQLCLIG	(167-186)
13	LCLIGCKPPIGEHWGKGSPC	(182-201)
14	KGSPCTNVAVNPGDCPPLEL	(197-216)
15	PPLELINTVIQDGDMVHTGF	(212-231)
16	VHTGFGAMDFTTLQANKSEV	(227-246)
17	NKSEVPLDICTSICKYPDYI	(242-261)
18	YPDYIKMVSEPYGDSLFFYL	(257-276)
19	LFFYLRREQMFVRHLFNRAY	(272-291)
20	FNRAGTVGENVPDDLYIKGS	(287-306)
21	YIKGSGSTANLASSNYFPTP	(302-321)
22	YFPTPSGSMVTSDAQIFNKP	(317-336)
23	IFNKPYWLQRAQGHNNGICW	(332-351)
24	NGICWGNQLFVTVVDTTRST	(347-366)
25	TTRSTNMSLCAAISTSETTY	(362-381)
26	SETTYKNTNFKEYLRHGEEY	(377-396)
27	HGEEYDLQFIFQLCKITLTA	(392-411)
28	ITLTADVMTYIHSMNSTILE	(407-426)
29	STILEDWNFGLQPPPGGTLE	(422-441)
30	GGTLEDTYRFVTQAIACQKH	(437-456)
31	ACQKHTPPAPKEDDPLKKYT	(452-471)
32	LKKYTFWEVNLKEKFSADLD	(467-486)
33	SADLDQFPLGRKFLLQAGLK	(482-501)
34	QAGLKAKPKFTLGKRKATPT	(497-516)
35	KATPTTSSTSTTAKRKKRKL	(512-531)
16a	VHTGFGAMDFTTLQ	(227-241)

Table 1b

PEPTIDE SEQUENCES OF 20 AA OVERLAPPING PEPTIDES CORRESPONDING TO THE HPV 16 L2 PROTEIN

No.	Peptide sequence	Range
36	RHKRSAKRTKRASATQLYKT	(2-21)
37	QLYKTCKQAGTCPPDIIPKV	(17-36)
38	IIPKVEGKTIAEQILQYGSM	(32-51)
39	QYGSMGVFFGGLGIGTGSGT	(47-66)
40	TGSGTGGRTGYIPLGTRPPT	(62-81)
41	TRPPTATDTLAPVRPPLTVD	(77-96)
42	PLTVDPVGPSDPSIVSLVEE	(92-111)
43	SLVEETSFIDAGAPTSVPSI	(107-126)
44	SVPSIPPDVSGFSITTSTDT	(122-141)
45	TSTDTTPAILDINNTVTTVT	(137-156)
46	VTTVTTHNNPTFTDPSVLQP	(152-171)
47	SVLQPPTPAETGGHFTLSSS	(167-186)
48	TLSSSTISTHNYEEIPMDTF	(182-201)
49	PMDTFIVSTNPNTVTSSTPI	(197-216)
50	SSTPIPGSRPVARLGLYSRT	(212-231)
51	LYSRTTQQVKVVDPAFVTTP	(227-246)
52	FVTTPTKLITYDNPAYEGID	(242-261)
53	YEGIDVDNTLYFSSNDNSIN	(257-276)
54	DNSINIAPDPDFLDIVALHR	(272-291)
55	VALHRPALTSRRTGIRYSRI	(287-306)
56	RYSRIGNKQTLRTRSGKSIG	(302-321)
57	GKSIGAKVHYYYDLSTIDPA	(317-336)
58	TIDPAEEIELQTITPSTYTT	(332-351)
59	STYTTTSHAASPTSINNGLY	(347-366)
60	NNGLYDIYADDFITDTSTTP	(362-381)
61	TSTTPVPSVPSTSLSGYIPA	(377-396)
62	GYIPANTTIPFGGAYNIPLV	(392-411)
63	NIPLVSGPDIPINITDQAPS	(407-426)
64	DQAPSLIPIVPGSPQYTIIA	(422-441)
65	YTIIADAGDFYLHPSYYMLRK	(437-456)
66	YMLRKRRKRLPYFFSDVSLAA	(452-471)

Table 2

ELISA titers against immunizing peptide and intact HPV16 capsids, "VLPs" of the five antisera that had the strongest reactivity with intact HPV16 VLPs. rb=rabbit antiserum. The other antisera were made in guinea pigs. Titer definition is >2 times background reactivity.

	HPV16 VLPs intact	Immunizing peptide
L1:3	1 : 5 400	1 : 5 400
L1:14	1 : 146 000	1 : 3 930 000
L1:16a	1 : 146 000	1 : 1 310 000
L1:20	1 : 16 200	1 : 437 000
L1: 20, rb.	1 : 146 000	1 : 1 310 000
L1:29	1 : 16 200	1 : 16 200

Table 3

Systematic optimisation of immunogen sequence to maximize ability to elicit papillomavirus-reactive antibodies. All the peptides are derived from HPV16 and those considered to be optimal immunogens are highlighted in bold. X=aminobutyric acid; Z=aminohexanoic acid.

Peptide synthesis number	Original peptide	Peptide titer	Intact virus titer	Disrupted virus titer	Sequence
A71-13	L1:3	66000	7300	7300	CATVYLPPVPVSKVVS
A71-23	L1:3	22000	7300	7300	PSEATVYLPPVPVSKVVSTD
A71-24	L1:3		7300	22000	CCCCCPSETAVYLPPVPVSKV VSTD
A71-25	L1:3	196000	7300	7300	GGGGPSETAVYLPPVPVSKVV STD
A71-26	**L1:3**	**66000**	**66000**	**22000**	**XPSEATVYLPPVPVSKVV STD**
A71-27	L1:3	196000	22000	22000	ZPSEATVYLPPVPVSKVVSTD
A71-28	**L1:29**	**66000**	**7300**	**2400**	**STILEDWNFGLQPPPGGT LE**
A71-30	L1:29	196000	7300	2400	GGGGSTILEDWNFGLQPPPGG TLE
A71-31	L1:29	66000	2400	2400	XSTILEDWNFGLQPPPGGTLE
A71-32	L1:29	66000	7300	2400	ZSTILEDWNFGLQPPPGGTLE
A71-18	L1:29	2400	810	810	CILEDWNFGLQPPPGGTLE
A71-33	L1:20	66000	7300	7300	FNRAGTVGENVPDDLYIKGS
A71-35	L1:20	7300	270	800	GGGGFNRAGTVGENVPDDLYI KGS
A71-36	L1:20	22000	2400	7300	XFNRAGTVGENVPDDLYIKGS
A71-37	L1:20	7300	270	800	ZFNRAGTVGENVPDDLYIKGS
A71-16	L1:20	2430	800	2400	CFNRAGTVGENVPDDLYIKGS
A71-17	**L1:20**	**22000**	**7300**	**7300**	**FNRAGTVGENVPDDLYIC**
A76-6	L1:20	22000	270	90	NRAGTVGENVPDDLYIXC
A76-7	L1:20	22000	2400	800	NRAGTVGENVPDDLYIC
A76-8	L1:20	22000	2400	800	RAGTVGENVPDDLYIC
A76-9	L1:20	22000	800	270	AGTVGENVPDDLYIC
A76-10	L1:20	65000	800	800	GTVGENVPDDLYIC
A76-11	L1:20	7300	270	90	TVGENVPDDLYIC
A16-14	L1:14	22000	800	270	KGSPCTNVAVNPGDCPPLEL
A71-14	L1:14	66000	800	270	KGSPCTNVAVNPGDSPPLEL
A71-15	L1:14	66000	800	800	KGSPSTNVAVNPGDCPPLEL
A76-12	**L1:14**	**66000**	**7300**	**7300**	**KGSPSTNVAVNPGDC**
A76-13	L1:14	7300	7300	800	SPSTNVAVNPGDCPPLEL
A76-14	L1:14	22000	2700	2700	PSTNVAVNPGDC

Table 4

AMINO ACID ALIGNMENTS FOR IDENTIFYING SURFACE-EXPOSED ANTIGENIC REGIONS OF CRPV, BPV AND HPV11 BY THEIR HOMOLOGY WITH THE SURFACE-EXPOSED REGIONS OF HPV16.

The surface-exposed epitopes of HPV16 and the maximally homologous peptides of other viruses are underlined.

L1: REGION:

HPV16: SLWLPSEATVYLPPVPVSKVVSTDEYVAR..

CRPV: AVWLSTQNKFYLPPQPVTKIPSTDEYVTR..

HPV11: WRPSDSTVYVPPPNPVSKVVATDAYVKR..

L1:14 REGION:

HPV16: GEHWGKGSPCTNVAVNPGDCPPLELINTVI..

CRPV: GEHWAQAKQCAEDPPQQTDCPPIELVNTVI..

L1:20 REGION:

HPV16: FVRHLFNRAGTVGENVPDDLYIKGSGSTAN..

CRPV: YARHFFSRAGGDKENVKSRAYIKRTQMQGE..

BPV: YVRHIWTRGGSEKEAPTTDFYLKNNKGDAT..

L1:29 REGION:

HPV16: IHSMNSTILEDWNFGLQPPPGGTLEDTYRF..

CRPV: LHSMNPTIIDNWQLSVSAQPSGTLEDQYRY..

Table 5

Induction of antibodies reactive with the capsid surface of other papillomaviruses by immunization with peptides homologous to the surface-exposed epitopes on HPV16.

Peptide synthesis number	Original peptide	Derived from:	Peptide titer	Intact virus titer	Disrupted virus titer	Sequence
A71-1	L1:3	HPV11	196000	2400	800	PSDSTVYVPPPNPVDKVVATD
A71-2	L1:3	CRPV	22000	7300	22000	STQNKFYLPPQPVTKIPSTD
A71-8	L1:20	CRPV	196000	800	300	FSRAGGDKENVKSRAYIKRT
A71-9	L1-20	BPV	196000	2400	2400	WTRGGSEKEAPTTDFYLKNN
A71-11	L1:29	CRPV	22000	300	800	PTIIDNWQLSVSAQPSGTLE
A80-4	L1:14	CRPV	7300	300	300	QAKQSAEDPPQQTDC

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CLAIMS

1. Vaccine against papillomavirus infection,
c h a r a c t e r i z e d in that it comprises as an immunizing component at least one peptide selected from the following groups of peptides a) to e)

a)

Ala Thr Val Tyr Leu Pro Pro Val Pro Val Ser Lys Val Val Ser
5 10 15

wherein Ala in position 1 may be substituted for Asn, Gly, Ser, or Thr,
Thr in position 2 may be substituted for Lys, Leu, Met or Gln,
Val in position 3 may be substitutde for Leu or Phe,
Leu in position 5 may be substituted for Val,
Val in position 8 may be substituted for Thr, Pro, Gln, Ala, or for the two amino-acid residues Pro Ala or Pro Asn,
Pro in position 9 may be substituted for Ser,
Ser in position 11 may be substituted for Ala or Thr,
Lys in position 12 may be substituted for Arg or Thr,
Val in position 13 may be substituted for Ile, Leu or omitted,
Val in position 14 may be substituted for Ile, Leu or Pro,
Ser in position 15 may be substituted for Asn, Ala, Pro or Thr,

b)

Gln Pro Leu Gly Val Gly Ile Ser Gly His Pro Leu Leu Asn Lys Leu Asp Asp Thr Glu
5 10 15 20

wherein Val in position 5 may be substituted for Leu, Ile or Ala,
Ile in position 7 may be substituted for any other amino-acid residue,
His in position 10 may be substituted for Asn,

Leu in position 12 may be substituted for Tyr or Phe,
Lys in position 15 may be substituted for Arg,
Leu in position 16 may be substituted for Gln, Tyr, Asp or Phe,
Asp in position 17 may be substituted for Glu or Asn,
Thr in position 19 may be substituted for Val,

c)

Lys Gly Ser Pro Cys Thr Asn Val Ala Val Asn Pro Gly Asp Cys Pro Pro Leu Glu Leu
5 10 15 20

wherein Lys, Gly and Ser in positions 1,2 and 3, respectively, may be individually omitted,
Pro in position 4 may be substituted for Leu, Ala, Val, Thr, Ile, Ser or Gln,
Cys in position 5 may be substituted for Ser,
Thr in position 6 may be substituted for Lys, Arg, Ala, Asn, Ser, or Gly,
Asn in position 7 may be substituted for Pro, Gln, Glu, Asn, Arg, Ser, Ala or Thr
Val in position 8 may be substituted for Asn, Asp, Pro, Thr, Ser, Ala, Arg or Gly,
Ala in position 9 may be substituted for Thr, Pro, Ser, Asn, Gln, Lys or Arg,
Val in position 10 may be substituted for Leu, Gln, Ser, Thr, Pro, Gly, Ile or omitted,
Asn in position 11 may be substituted for Ala, Ser, Gln, Thr, Arg, Pro, Val, Lys or omitted,
Pro in position 12 may be substituted for Ala, Asn, Gln,, Val, Arg, Thr, Leu, Asp, Ser or omitted,
Asp in position 14 may be substituted for Glu,
Cys, Pro, Pro, Leu, Glu and Leu in positions 15, 16, 17, 18, 19 and 20, respectively, may be individually omitted,

d)

Arg Ala Gly Thr Val Gly Glu Asn Val Pro Asp Asp Leu Tyr Ile
5 10 15

wherein Arg in position 1 may be substituted for Lys or Leu,
Ala in position 2 may be substituted for Gln, Gly, Leu or Ser,
Gly in position 3 may be substituted for Ser or Val,
Thr in position 4 may be substituted for Ala, Met, Val, Asp, Lys, Ser, Gly, Asn or Glu,
Val in position 5 may be substituted for Met, Leu, Ile, Ala or Thr,
Glu in position 7 may be substituted for Asp,
Asn in position 8 may be substituted for Pro, Thr, Ala, Lys, Asp, Gln, Glu or Ser,
Val in position 9 may be substituted for Ile or Leu,
Asp in position 11 may be substituted for Asn, Glu, Thr, Ser, Ala, Gln or Gly,
Asp in position 12 may be substituted for Glu, Ala, Thr, Ser or Gln,
Leu in position 13 may be substituted for Met, Phe or Tyr,
Tyr in position 14 may be substituted for Ile, Leu, Val or Met,
Ile in position 15 may be substituted for Thr, Leu, Ile, Val, Trp or Phe,

and

e)

Ser Thr Ile Leu Glu Asp Trp Asn Phe Gly Leu Gln Pro Pro Pro Gly Gly Thr Leu Glu
5 10 15 20

wherein Ser in position 1 may be substituted for Pro, Asn, Lys, Ala or Thr,
Thr in position 2 may be substituted for Asp, Asn, Ser, Ala, Gln, Arg or Gly,
Ile in position 3 may be substituted for Leu,
Leu in position 4 may be substituted for Ile,
Glu in position 5 may be substituted for Asp,

Asp in position 6 may be substituted for Glu, Gln, Gly or Asn,
Asn in position 8 may be substituted for Gln,
Phe in position 9 may be substituted for Val or Ile,
Gly in position 10 may be substituted for Ala, Ser or Lys,
Leu in position 11 may be substituted for Ile or Val,
Gln in position 12 may be substituted for Thr, Ala, Val, Pro, Ser or Gly,
Pro in position 13 may be substituted for Ala, Leu or Thr,
Pro in position 14 may be substituted for Ala or Val,
Pro in position 15 may be substituted for Gln,
Gly in position 16 may be substituted for Ser, Thr, Ala or Asn,
Gly in position 17 may be substituted for Ser, Thr or Ala,
Thr in position 18 may be substituted for Ser,
Glu in position 20 may be substituted for Gln or Val.

2. Vaccine according to claim 1, wherein said vaccine comprises at least two immunizing components selected from at least two different groups of peptides a) to e).

3. Vaccine according to claim 1 or 2, wherein said immunizing component is chosen from the peptides

a)

Ala Thr Val Tyr Leu Pro Pro Val Pro Val Ser Lys Val Val Ser
5 10 15

b)

Gln Pro Leu Gly Val Gly Ile Ser Gly His Pro Leu Leu Asn Lys Leu Asp Asp Thr Glu
5 10 15 20

c)

Lys Gly Ser Pro Cys Thr Asn Val Ala Val Asn Pro Gly Asp Cys Pro Pro Leu Glu Leu
5 10 15 20

c1)

Pro Ser Thr Asn Val Ala Val Asn Pro Gly Asp
5 10

d)

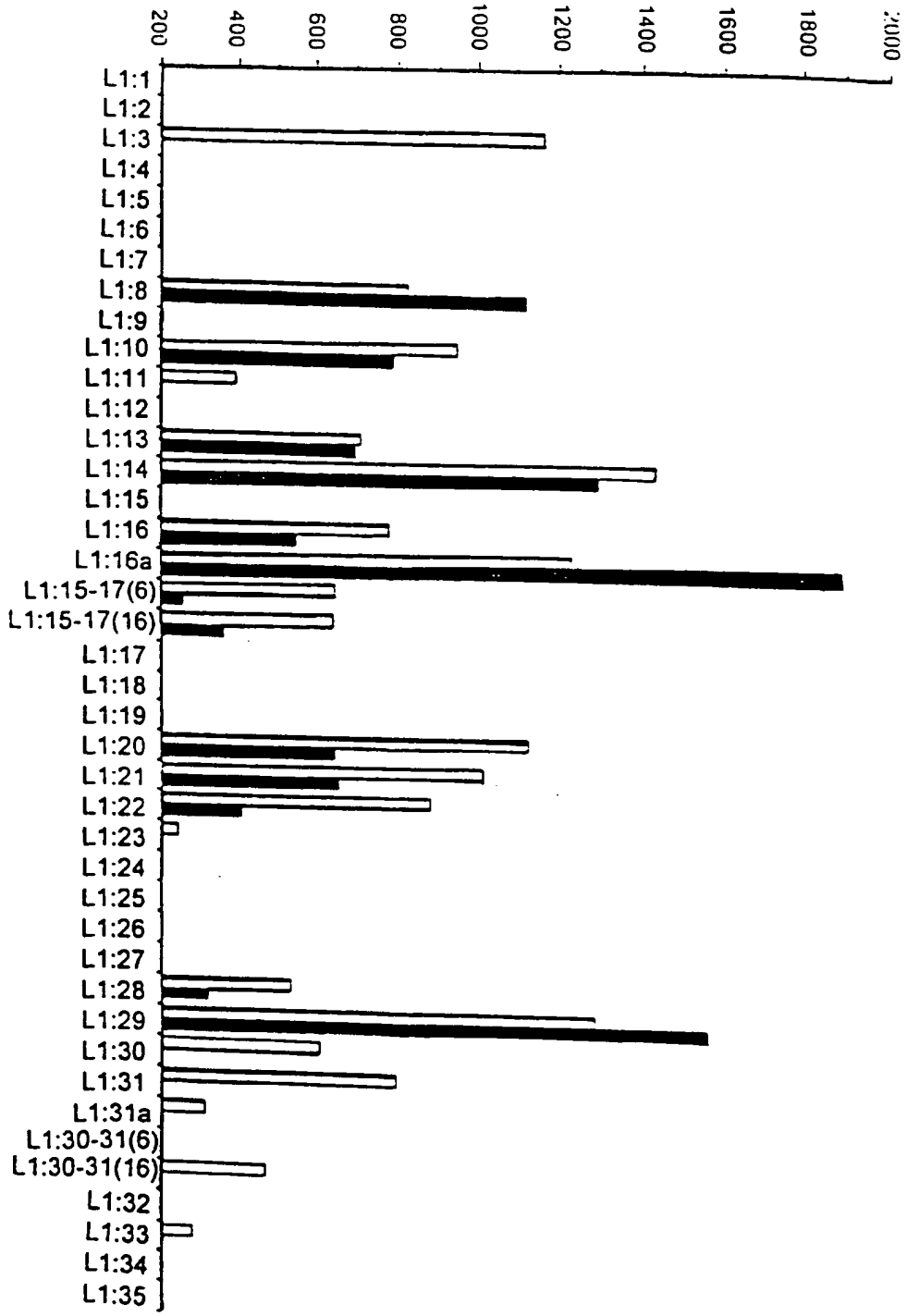
Arg Ala Gly Thr Val Gly Glu Asn Val Pro Asp Asp Leu Tyr Ile
5 10 15

e)

Ser Thr Ile Leu Glu Asp Trp Asn Phe Gly Leu Gln Pro Pro Pro Gly Gly Thr Leu Glu
5 10 15 20

4. Vaccine according to any one of claims 1-3, wherein said peptide(s) is (are) coupled to a carrier or is (are) multimer forms of the peptide(s).

5. Vaccine according to any one of claims 1-4, which additionally comprises an adjuvant.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400
600
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1000
1200
1400
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1800
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L1:1
L1:2
L1:3
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L1:30
L1:31
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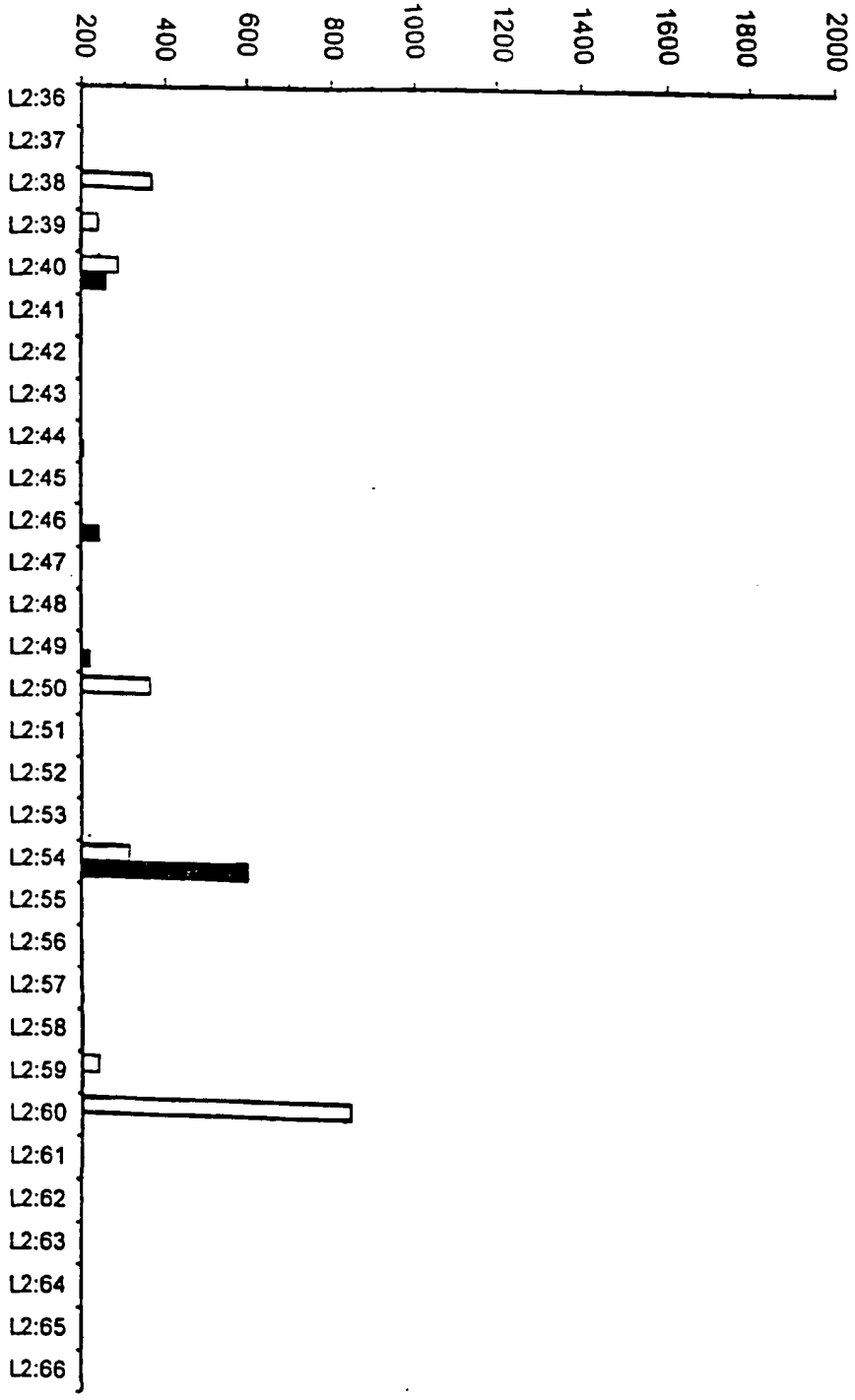
L1:32
L1:33
L1:34
L1:35

FIG. 1a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400
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1000
1200
1400
1600
1800
2000
L2:36
L2:37
L2:38
L2:39
L2:40
L2:41
L2:42
L2:43
L2:44
L2:45
L2:46
L2:47
L2:48
L2:49
L2:50
L2:51
L2:52
L2:53
L2:54
L2:55
L2:56
L2:57
L2:58
L2:59
L2:60
L2:61
L2:62
L2:63
L2:64
L2:65
L2:66

FIG. 1b

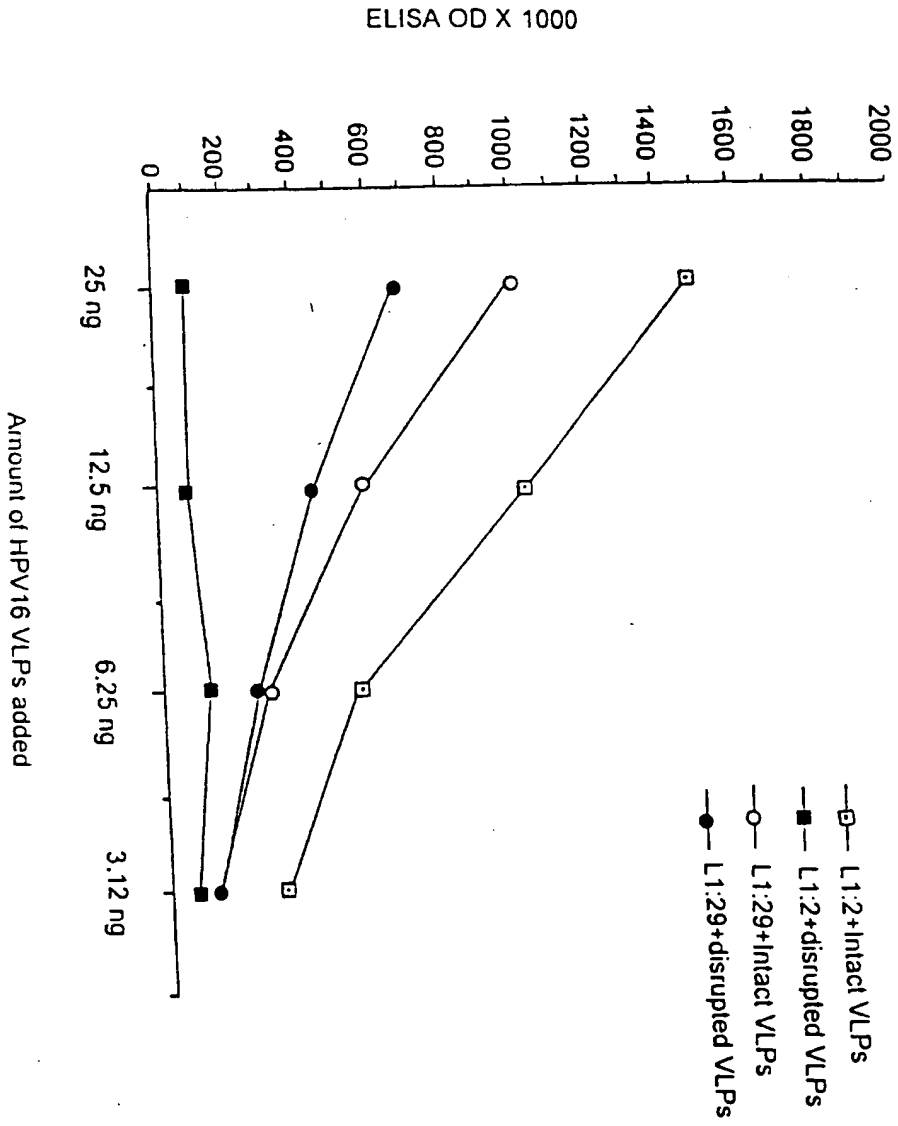
ELISA OD X 1000
0
200
400
600
800
1000
1200
1400
1600
1800
2000
25 ng
12.5 ng
6.25 ng
3.12 ng
Amount of HPV16 VLPs added
L1:2+Intact VLPs
L1:2+disrupted VLPs
L1:29+Intact VLPs
L1:29+disrupted VLPs

FIG. 2

INTERNATIONAL SEARCH REPORT

International application No.
PCT/SE 96/00533

A. CLASSIFICATION OF SUBJECT MATTER

IPC6: A61K 39/12, C07K 14/025
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC6: A61K, C07K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

SE,DK,FI,NO classes as above

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

MEDLINE, SCISEARCH

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 9302184 A1 (THE UNIVERSITY OF QUEENSLAND), 4 February 1993 (04.02.93), page 17 - page 29, figure 12, claim 39	1-5
	--	
X	Journal of General Virology, Volume 71, 1990, George Strang et al, "Human T cell responses to human papillomavirus type 16 L1 and E6 synthetic peptides: identification of T cell determinants, HLA-DR restrict ion and virus type specificity", page 423 - page 431, see tables	1-5
	--	

[X] Further documents are listed in the continuation of Box C. [X] See patent family annex.

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"E" erlier document but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance: the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance: the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
16 July 1996	27 -08- 1996
Name and mailing address of the ISA/ Swedish Patent Office Box 5055, S-102 42 STOCKHOLM Facsimile No. +46 8 666 02 86	Authorized officer Carl-Olof Gustafsson Telephone No. +46 8 782 25 00

INTERNATIONAL SEARCH REPORT

International application No.
PCT/SE 96/00533

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	Journal of general virology, Volume 189, 1992, Jian Zhou et al, "Definition of linear antigenic regions of HPV 16 L1 capsid protein using synthetic virion-like particles", page 592 - page 599, see table 1, figure 2 and discussion	1-5
	--	
A	EP 0451550 A2 (BEHRINGWERKE), 16 October 1991 (16.10.91), see example 3 and claims 5-9 and 14	1-5
	--	
A	WO 9501374 A1 (BRITISH TECHNOLOGY GROUP LIMITED), 12 January 1995 (12.01.95), page 2, line 23 - page 3, line 24; page 5, line 20 - page 6, line 20, see table 1 and the claims	1-5
	--	
A	WO 9118294 A1 (MEDSCAND AB), 28 November 1991 (28.11.91), see page 22 - page 25	1-5
	--	
A	Int. J. Cancer, Volume 45, 1990, Joakim Dillner et al, "Mapping of linear epitopes of human papillomavirus type 16: the L1 and L2 open reading frames", page 529 - page 535, see table 1	1-5
	--	
A	The Cancer Journal, Volume 5, No 4, July 1992, J. Dillner, "Immunobiology of papillomavirus. Prospects for vaccination" page 182 - page 187	1-5
	--	
X	Proc. Natl. Acad. Sci., Volume 89, Sept 1992, Hans J. Strauss et al, "Induction of cytotoxic T lymphocytes with peptides in vitro: Identification of candidate T-cell epitopes in human papilloma virus", page 7871 - page 7875, See Table 3, L1-(141-155) L1-(201-215), and table 2	1-5
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INTERNATIONAL SEARCH REPORT

International application No.
PCT/SE 96/00533

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	Arch Virol, Volume 140, 1995, J. E. Ramesar et al, "Sequence variation in the L1 gene of human papillomavirus type 16 from Africa", page 1863 - page 1870, see page 1867 - page 1868	1,3
A	Journal of General Virology, Volume 75, 1994, Peter Pushko et al, "Sequence variation in the capsid protein genes of human papillomavirus type 16", page 911 - page 916, see pages 913-914	1,3
A	Journal of General Virology, Volume 71, 1990, M. Müller et al, "Identification of seroreactive regions of the human papillomavirus type 16 proteins E4, E6, E7 and L1", page 2709 - page 2717, see pages 2712-13, (clone"809(L1)"peptide)	1,3
A	Journal of Medical Virology, Volume 45, 1995, P. Le Cann et al, "Detection of Antibodies to L1, L2, and E4 Proteins of Human Papillomavirus Types 6, 11, and 16 by ELISA Using Synthetic Peptides", page 410 - page 414, see Table I peptide"16L1a"	1,3
A	Gynecologic Oncology, Volume 55, 1994, J.F. Hines et al, "Role of Conformational Epitopes Expressed by Human Papillomavirus Major Capsid Proteins in the Serologic Detection of Infection and Prophylactic Vaccination", page 13 - page 20, see page 19, right column	1-5
A	Gynecologic oncology, Volume 55, 1994, J.F. Hines et al, "Role of Conformational Epitopes Expressed by Human Papillomavirus Major Capsid Proteins in the Serologic Detection of Infection and Prophylactic Vaccination", page 13 - page 20, see page 19, right column	1-5

INTERNATIONAL SEARCH REPORT

International application No.
PCT/SE 96/00533

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	Vaccine, Volume 11, No 6, 1993, J. Cason et al, "Towards vaccines against human papillomavirus type-16 genital infections", page 603, see in particular page 606 right column - page 607	1-5

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INTERNATIONAL SEARCH REPORT

Inte...ational application No.
PCT/SE 96/00533

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.: 1 c-e, 3 c-e
because they relate to subject matter not required to be searched by this Authority, namely:
Due to the large number of variable positions in the amino acid chains and the variability in the choice of amino acids in each of these positions, a full evaluation of the relevance of the state of the art literature has not been made. The search has therefore essentially been restricted to positions and amino acids supported by the examples.

2. [ ] Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. [ ] As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest
- [ ] The additional search fees were accompanied by the applicant's protest.
- [ ] No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

Information on patent family members

31/07/96

International application No.
PCT/SE 96/00533

Patent document cited in search report	Publication date	Patent family member(s)		Publication date
WO-A1- 9302184	04/02/93	AU-B-	651727	28/07/94
		EP-A-	0595935	11/05/94
		JP-T-	7505042	08/06/95
EP-A2- 0451550	16/10/91	AU-B-	650868	07/07/94
		AU-A-	7351591	26/09/91
		CA-A-	2038581	21/09/91
		JP-A-	4217998	07/08/92
WO-A1- 9501374	12/01/95	AU-A-	7040594	24/01/95
		EP-A-	0706533	17/04/96
		GB-A-	2279651	11/01/95
		GB-D-	9412915	00/00/00
WO-A1- 9118294	28/11/91	AU-B-	668499	09/05/96
		AU-A-	7889091	10/12/91
		CA-A-	2040849	20/10/92
		CA-A-	2082658	12/11/91
		EP-A-	0594613	04/05/94
		JP-T-	6501542	17/02/94